formation of disulfide cross-links since there would be an increased amount of ionized thiol at the higher pH values (Kamat, Tolman, & Brown, 1996). A mAb that was freeze-dried without any lyoprotectants had about 35% aggregate after storage as a freeze-dried preparation for 1 year at 30 °C (Andya, Hsu, & Shire, 2003). Separation of the aggregates by sizing chromatography and determination of weight average molecular weights with an online light scattering detector resulted in determined molecular weights consistent with monomers, dimers, and trimers (Figure 3.13(a)). Reduced versus nonreduced sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) conclusively proved that these aggregates were the result of formation of intermolecular disulfide bonds (Figure 3.13(b)). In another study, spray drying of an anti-IgE IgG1 mAb resulted in formation of disulfide cross-links (Andya et al., 1999). Often solid-state formulations using drying technology such as freeze-drying or spray drying without sufficient protective excipients can result in disulfide exchange since the average distance between the mAbs is greatly reduced compared to aqueous formulations. Since the reaction requires two Cys residues the rate of disulfide formation generally is inversely related to the distance between the residues. It has been demonstrated that the oxidation of Cys to cystine (disulfide link) decreases as the distance between the residues increases (Barron, Miller, & Kalnitsky, 1947; Overberger & Ferraro, 1962).

Nonenzymatic peptide fragmentation

The peptide bond generally is very stable requiring highly acidic conditions and high temperature to fragment. Under mild acidic conditions, where Asp residues are not ionized, cleavage of the Asp peptide bonds occurs at ~100× the rate of other peptide bonds (Schultz, 1967), and occurs more frequently at Asp–Gly and Asp–Pro sites in proteins (Powell, 1996). Possible pathways for cleavage at Asp peptide bonds have been proposed (Inglis, 1983). It has also been reported that fragmentation at Asn and Asp occurs through the succinimide intermediate that is formed during deamidation of proteins (Geiger & Clarke, 1987; Klotz & Thomas, 1993; Voorter, Dehaardhoekman, Vandenoetelaar, Bloemendal, & Dejong, 1988). The cleavage of peptide chains at an Asp residue via a succinimide intermediate has been studied using theoretical computations (Catak, Monard, Aviyente, & Ruiz-Lopez, 2008). Although deamidation of the Asn predominates cleavage at Asp sites, it was proposed that if deamidation via the succinimide intermediate is prevented by protein tertiary structure, fragmentation may be a competing pathway. It was also shown that the activation barrier for cleavage at Asp residues is ~10 kcal/mol lower than for Asn, suggesting that fragmentation usually occurs after the Asn residue has deamidated into an Asp residue. Based on the theoretical computations a mechanism for fragmentation at Asp through a succinimide intermediate was proposed (Figure 3.14).

Fragmentation in mAbs

Fragmentation of peptide chains has been reported for several mAbs. Fragments were observed after an antibody against human cytomegalovirus was stored for 14 days at 37 °C at pH 4 and pH 10. These fragments were detected using reducing and